WEST Search History

DATE: Friday, February 21, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,DWPI; PLUR=YES; OP=ADJ			
L1	bertelli-F$.in. or brown-J$.in. or Dissanayake-V$.in. or suman-$.in. or gee-N$.in.	3167	L1
L2	cerbral cortical voltage dependent calcium channel	0	L2
L3	voltage-dependent calcium channel	140	L3
L4	l1 and l3	1	L4
L5	5429921.pn.	2	L5
DB=USPT,EPAB,DWPI; PLUR=YES; OP=ADJ			
L6	alpha-2 beta-1 subunit	0	L6
L7	l1 and calcium channel	4	L7
L8	alpha-2 delta-1 subunit	0	L8
L9	alpha-2 delta subunit	0	L9
L10	cerbral cortical	3	L10
L11	l3 and (compound or ligand)	132	L11
L12	l11 and screening	101	L12
L13	L12 and binding	97	L13
L14	flashplate assay or flashplate	68	L14
L15	L14 and l13	0	L15
L16	L14 and l3	0	L16
L17	alpha near subunit	2440	L17
L18	L17 and l13	54	L18
L19	soluble recombinant calcium channel	0	L19
L20	soluble near calcium channel	3	L20
DB=USPT,DWPI; PLUR=YES; OP=ADJ			
L21	secret$2 near soluble near calcium channel	0	L21

END OF SEARCH HISTORY

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(FILE 'HOME' ENTERED AT 14:13:19 ON 21 FEB 2003)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 14:14:44 ON 21 FEB 2003
L1         41453 S BERTELLI F?/AU OR BROWN J?/AU OR DISSANAYAKE V?/AU OR SUMAN
?
L2             4 S L1 AND VOLTAGE DEPENDENT CALCIUM CHANNEL
L3             0 S CERBRAL CORTICAL (S) VOLTAGE DEPENDENT CALCIUM CHANNEL
L4             0 S CERBRAL CORTICAL AND CALCIUM CHANNEL
L5        261737 S CEREBRAL AND (CORTEX OR CORTICAL)
L6            88 S L5 AND VOLTAGE DEPENDENT CALCIUM CHANNEL
L7             0 S L6 AND RECOMBINANT CALCIUM CHANNEL
L8             2 S L6 AND ALPHA.2.DELTA. SUBUNIT

     FILE 'STNGUIDE' ENTERED AT 14:25:56 ON 21 FEB 2003
L9             0 S L5 AND ALPHA.2.DELTA. SUBUNIT
L10            0 S VOLTAGE DEPENDENT CALCIUM CHANNNEL

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 14:28:49 ON 21 FEB 2003
L11           19 S L5 AND ALPHA.2.DELTA. SUBUNIT
L12            9 DUP REM L11 (10 DUPLICATES REMOVED)
L13           19 S VOLTAGE DEPENDENT CALCIUM CHANNEL AND ALPHA.2.DELTA. SUBUNIT
L14           15 DUP REM L13 (4 DUPLICATES REMOVED)
L15            0 S L13 AND FLASHPLATE
L16           70 S FLASHPLATE
L17            3 S L16 AND CALCIUM CHANNEL
L18         1681 S SPA ASSAY OR NICKEL FLASHPLATE ASSAY OR FILTER BINDING
ASSAY
L19            1 S L18 AND CALCIUM CHANNEL
L20            0 S L13 AND LIGAND
L21            3 S (L13 OR L11) AND COMPOUND
L22            7 S (L13 OR L11) AND LIGAND
L23            0 S (L13 OR L11) AND LEVEL AND BINDING

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Gabapentin (neurontin) and S-(+)-3-isobutylgaba represent a novel class of selective antihyperalgesic agents.

AUTHOR(S): Field, M. J.; Oles, R. J.; Lewis, A. S.; McCleary, S.; Hughes, J.; Singh, L. (1)
CORPORATE SOURCE: (1) Dep. Biol., Parke-Davis Neurosci. Res. Centre, Cambridge Univ. Forvie Site, Robinson Way, Cambridge CB2 2QB UK

SOURCE: British Journal of Pharmacology, (1997) Vol. 121, No. 8, pp. 1513-1522.
ISSN: 0007-1188.
DOCUMENT TYPE: Article
LANGUAGE: English

AB 1. Gabapentin (neurontin) is a novel antiepileptic agent that binds to the **alpha-2-delta subunit** of voltage-dependent calcium channels. The only other compound known to possess affinity for this recognition site is the (S)-(+)-enantiomer of 3-isobutylgaba. However, the corresponding (R)-(-)-enantiomer is 10 fold weaker. The present study evaluates the activity of gabapentin and the two enantiomers of 3-isobutylgaba in formalin and carrageenan-induced inflammatory pain models. 2. In the rat formalin test, S-(+)-3-isobutylgaba (1- 100 mg kg-1) and gabapentin (10- 300 mg kg-1) dose-dependently inhibited the late phase of the nociceptive response with respective minimum effective doses (MED) of 10 and 30 mg kg-1, s.c. This antihyperalgesic action of gabapentin was insensitive to naloxone (0.1-10.0 mg kg-1, s.c.). In contrast, the R-(-)-enantiomer of 3-isobutylgaba (1100 mg kg-1) produced a modest inhibition of the late phase at the highest dose of 100 mg kg-1. However, none of the compounds showed any effect during the early phase of the response. 3. The s.c. administration of either S-(+)-3-isobutylgaba (1-30 mg kg-1) or gabapentin (10100 mg kg-1), after the development of peak carrageenan-induced thermal hyperalgesia, dose-dependently antagonized the maintenance of this response with MED of 3 and 30 mg kg-1, respectively. Similar administration of the two compounds also blocked maintenance of carrageenan-induced mechanical hyperalgesia with MED of 3 and 10 mg kg-1, respectively. In contrast, R-(-)-3-isobutylgaba failed to show any effect in the two hyperalgesia models. 4. The intrathecal administration of gabapentin dose-dependently (1-100 mu-g/animal) blocked carrageenan-induced mechanical hyperalgesia. In contrast, administration of similar doses of gabapentin into the inflamed paw was ineffective at blocking this response. 5. Unlike morphine, the repeated administration of gabapentin (100 mg kg-1 at start and culminating to 400 mg kg-1) over 6 days did not lead to the induction of tolerance to its antihyperalgesic action in the formalin test. Furthermore, the morphine tolerance did not cross generalize to gabapentin. The s.c. administration of gabapentin (10-300 mg kg-1), R-(-) (3-100 mg kg-1) or S-(+)-3-isobutylgaba (3100 mg kg-1) failed to inhibit gastrointestinal motility, as measured by the charcoal meat test in the rat. Moreover, the three compounds (1-100 mg kg-1, s.c.) did not generalize to the morphine discriminative stimulus. Gabapentin (30-300 mg kg-1) and S-(+)-isobutylgaba (1-100 mg kg-1) showed sedative/ataxic properties only at the highest dose tested in the rota-rod apparatus. 6. Gabapentin (30-300 mg kg-1, s.c.) failed to show an antinociceptive action in transient pain models. It is concluded that gabapentin represents a novel class of antihyperalgesic agents.

L14 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:141190 BIOSIS
DOCUMENT NUMBER: PREV199799440393
TITLE: Characterization of interaction domains between the **voltage-dependent calcium channel** alpha-2-delta and alpha-1 subunits.
AUTHOR(S): Gurnett, Christina A. (1); Felix, Ricardo; Campbell, Kevin P.
CORPORATE SOURCE: (1) Howard Hughes Med. Inst., Univ. Iowa Coll. Med., Iowa City, IA 52242 USA
SOURCE: Biophysical Journal, (1997) Vol. 72, No. 2 PART 2, pp. A23.

Meeting Info.: 41st Annual Meeting of the Biophysical Society New Orleans, Louisiana, USA March 2-6, 1997
ISSN: 0006-3495.
DOCUMENT TYPE: Conference; Abstract
LANGUAGE: English

Cloning and deletion mutagenesis of the .alpha.2.delta. calcium channel subunit from porcine **cerebral cortex**: Expression of a soluble form of the protein that retains [3H]gabapentin binding activity.

AUTHOR: Browns J.P.; Gee N.S.
CORPORATE SOURCE: J.P. Browns, Parke-Davis Neuroscience Res. Centre, Cambridge University Forvie Site, Robinson Way, Cambridge CB2 2QB, United Kingdom. Jason.Brown@wl.com
SOURCE: Journal of Biological Chemistry, (25 Sep 1998) 273/39 (25458-25465).
Refs: 54
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The anti-epileptic, anti-hyperalgesic, and anxiolytic agent gabapentin (1-(aminomethyl)-cyclohexane acetic acid or Neurontin) has previously been shown to bind with high affinity to the .**alpha.2.delta. subunit** of voltage-dependent calcium channels (Gee, N. S., Brown, J.P., Dissanayake, V. U. K., Offord, J., Thurlow, R., and Woodruff, G.N. (1996) J. Biol. Chem. 271, 5768-5776). We report here the cloning, sequencing, and deletion mutagenesis of the .**alpha.2.delta. subunit** from porcine brain. The deduced protein sequence has a 95.9 and 98.2% identity to the rat and human neuronal .alpha.2.delta. sequences, respectively. [3H]Gabapentin binds with a K(D) of 37.5 .+-. 10.4 nM to membranes prepared from COS-7 cells transfected with wild-type porcine .alpha.2.delta. cDNA. Six deletion mutants (B-G) that lack the .delta. polypeptide, together with varying amounts of the .alpha.2 component, failed to bind [3H]gabapentin. C-terminal deletion mutagenesis of the .delta. polypeptide identified a segment (residues 960-994) required for correct assembly of the [3H]gabapentin binding pocket. Mutant L, which lacks the putative membrane anchor in the sequence, was found in both membrane-associated and soluble secreted forms. The soluble form was not proteolytically cleaved into separate .alpha.2 and .delta. chains but still retained a high affinity (K(D) = 30.7 .+-. 8.1 nM) for [3H]gabapentin. The production of a soluble .alpha.2.delta. mutant supports the single transmembrane model of the .**alpha.2.delta. subunit** and is an important step toward the large-scale recombinant expression of the protein.

Mechanisms of action of gabapentin

AUTHOR(S): **Brown, J. P.**; Boden, P.; Singh, L.; Gee, N. S.
CORPORATE SOURCE: Parke-Davis Neuroscience Research Centre, Cambridge University, Cambridge, CB2 2QB, UK

SOURCE: Reviews in Contemporary Pharmacotherapy (1996), 7(5), 203-214
CODEN: RCPHFW; ISSN: 0954-8602
PUBLISHER: Marius Press
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with over 80 refs. Gabapentin [1-(aminomethyl)-cyclohexaneacetic acid; Neurontin.RTM.] is an antiepileptic drug that is structurally related to .gamma.-amino-butyric acid (GABA). It has a unique spectrum of activity in animal seizure models and has demonstrable efficacy in patients with refractory epilepsy. Although designed as a GABA-mimetic, gabapentin does not interact with any of the known pharmacol. sites on either the GABAA or GABAB receptor, nor does it block GABA uptake or inhibit the GABA-metabolizing enzyme, GABA transaminase. Gabapentin has been shown to elevate GABA levels in various brain regions of the rat but the relevance of this effect to the anticonvulsant activity of the drug remains unclear. Some electrophysiol. studies suggest that gabapentin may act as a partial agonist at the glycine modulatory site of the NMDA receptor. The reversal of the anticonvulsant effects of gabapentin in animal seizure models by D-serine, an agonist at the glycine modulatory site, further supports this notion. However, radioligand binding studies provide no evidence for any direct interaction of gabapentin with the NMDA receptor. A novel high affinity binding site for [3H]gabapentin has been identified in rat, mouse and pig brain membranes. While none of the front-line antiepileptic drugs has a high affinity for this site, several 3-substituted analogs of GABA and neutral amino acids, such as L-leucine, potently inhibit [3H]gabapentin binding. The binding protein has recently been purified to homogeneity and identified as the .alpha.2.delta. subunit of a **voltage-dependent calcium channel** (VDCC). Finally, behavioral studies suggest that gabapentin possesses not only antiepileptic but also anxiolytic and antinociceptive/anti-hyperalgesic properties. Further expts. are required to det. which, if any, of these behavioral effects are related to the interaction of gabapentin with GABAergic systems, NMDA receptors or neuronal VDCCs.

Spermine modulation of specific [3H]-gabapentin binding to the detergent-solubilized porcine **cerebral cortex** alpha 2 delta calcium channel subunit.

AUTHOR: Dissanayake V U; Gee N S; Brown J P; Woodruff G N
CORPORATE SOURCE: Parke-Davis Neuroscience Research Centre, Cambridge University Forvie Site.

SOURCE: BRITISH JOURNAL OF PHARMACOLOGY, (1997 Mar) 120 (5) 833-40.
Journal code: 7502536. ISSN: 0007-1188.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970514
Last Updated on STN: 19970514
Entered Medline: 19970502

AB 1. Recent studies have identified the [3H]-gabapentin-binding protein, purified from porcine **cerebral cortical** membranes, as the **alpha 2 delta subunit** of voltage-sensitive calcium channels (Gee et al., 1996). The present study investigates the influence of the polyamine spermine on specific [3H]-gabapentin binding to detergent-solubilized porcine **cerebral cortical** membranes. 2. Spermine, spermidine, 1,10 diaminodecane, Mg2+ and Zn2+, all divalent cations, displaced [3H]-gabapentin binding to detergent-solubilized membranes in a concentration-dependent manner with a maximal inhibition of 65-75%. Radioligand binding studies showed that spermine did not directly interact with the [3H]-gabapentin-binding site. Spermine inhibited [3H]-gabapentin binding by interacting with a polyamine-sensitive allosteric site on the membrane protein. The steep concentration-dependence of spermine inhibition of [3H]-gabapentin binding may suggest multi-site co-operativity. 3. Prolonged dialysis of **cerebral cortical** membranes and Tween 20-solubilized membranes resulted in a > 2.0 fold increase in [3H]-gabapentin binding. The increase in binding was due to the removal of a heat stable, low molecular weight (< 12,000Da) endogenous molecule which influences [3H]-gabapentin binding competitively. 4. Dialysis of detergent-solubilized **cerebral cortical** membranes also resulted in a decrease in the maximum inhibition of [3H]-gabapentin binding by spermine. Since the rates of the increase in [3H]-gabapentin binding and the loss of the ability of spermine to inhibit [3H]-gabapentin binding on dialysis were different it was inferred that a second endogenous ligand was removed during dialysis. 5. During initial steps of purification of the [3H]-gabapentin-binding protein there was a decrease in the maximum inhibition of [3H]-gabapentin binding by spermine. The loss of the second endogenous molecule during initial purification would reasonably explain the reduction in inhibition of binding by spermine. However, spermine stimulation of [3H]-gabapentin binding to material that eluted from the gel-filtration column later in the purification scheme does not appear to be due to removal of a dialysable endogenous factor or to the dissociation of other calcium channel subunit(s). 6. Adding back dialysate, before or after boiling, to detergent solubilized membranes resulted in a dose-dependent restoration of the inhibition of [3H]-gabapentin binding and of the maximal inhibition [3H]-gabapentin binding by spermine. This result is consistent with the re-addition of two endogenous heat stable ligands. 7. The findings that [3H]-gabapentin binding to the pure **alpha 2 delta subunit** was stimulated

by spermine indicates that the **alpha 2 delta subunit** of voltage-sensitive calcium channels bears a modulatory spermine site. Such a spermine site has not been identified before. Spermine stimulation of [3H]-gabapentin binding to the purified protein was reversed to inhibition after adding back dialysate. Thus the inhibitory spermine effect in membranes is also probably due to one or more modulatory sites on the **alpha 2 delta subunit**.

Isolation of the [3H]gabapentin-binding protein/alpha 2 delta Ca2+ channel subunit from porcine brain: development of a radioligand binding assay for alpha 2 delta subunits using [3H]leucine.

AUTHOR: Brown J P; Dissanayake V U; Briggs A R; Milic M R; Gee N S
CORPORATE SOURCE: Parke-Davis Neuroscience Research Centre, Cambridge University Forvie Site, Cambridge, United Kingdom.

SOURCE: ANALYTICAL BIOCHEMISTRY, (1998 Jan 15) 255 (2) 236-43.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980410
Last Updated on STN: 19980410
Entered Medline: 19980327

AB The novel antiepileptic agent gabapentin (Neurontin) binds with high affinity to the **alpha 2 delta subunit** of a voltage-dependent Ca2+ channel. We report here a simple purification scheme for detergent-solubilized alpha 2 delta subunits from porcine brain. This involves sequential chromatography on Q-Sepharose, Cu(2+)-charged iminodiacetic acid-Sepharose, wheat germ lectin-agarose, and Mono Q. The purified protein was essentially homogeneous by SDS-polyacrylamide gel electrophoresis with a subunit Mr of 145,000. Using [3H] gabapentin as the radiolabeled tracer and (S)-3-isobutyl gamma-aminobutyric acid to define nonspecific binding, the overall purification factor was 2760-fold and the apparent yield 26.6%. We also developed and validated a novel binding assay for alpha 2 delta Ca2+ channel subunits using the ligand pair L-[3H leucine/L-isoleucine. Even in binding assays of crude brain membrane fractions, [3H]leucine proved to be remarkably stable and specific for the alpha 2 delta Ca2+ channel subunit.

[3H]Leucine offers several advantages over custom-labeled [3H]gabapentin: it has a higher specific activity, is relatively inexpensive, and is available from commercial sources.